

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

WRITTEN OPINION OF THE
INTERNATIONAL PRELIMINARY
EXAMINING AUTHORITY
(PCT Rule 66)

		Date of mailing (day/month/year) <i>27.10.2005</i>
Applicant's or agent's file reference 11343P5 WO/JM	REPLY DUE	within 2 month(s) from the above date of mailing
International application No. PCT/GB2004/004637	International filing date (day/month/year) 04.11.2004	Priority date (day/month/year) 14.11.2003
International Patent Classification (IPC) or both national classification and IPC A01M13/00, A01N53/00		
<p>Applicant RECKITT BENCKISER (AUSTRALIA) PTY LIMITED</p>		
<p>1. <input checked="" type="checkbox"/> The written opinion established by the International Searching Authority: <input checked="" type="checkbox"/> is <input type="checkbox"/> is not considered to be a written opinion of the International Preliminary Examining Authority <i>2005</i></p> <p>2. This first report contains indications relating to the following items:</p> <ul style="list-style-type: none"> <input checked="" type="checkbox"/> Box No. I Basis of the opinion <input type="checkbox"/> Box No. II Priority <input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability <input type="checkbox"/> Box No. IV Lack of unity of invention <input checked="" type="checkbox"/> Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement <input checked="" type="checkbox"/> Box No. VI Certain documents cited <input type="checkbox"/> Box No. VII Certain defects in the international application <input type="checkbox"/> Box No. VIII Certain observations on the international application <p>3. The applicant is hereby invited to reply to this opinion.</p> <p>When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(e).</p> <p>How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3.</p> <p>Also: For the form and the language of the amendments, see Rules 66.8 and 66.9. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.6. For an additional opportunity to submit amendments, see Rule 66.4.</p> <p>If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.</p> <p>4. The final date by which the international preliminary report on patentability (Chapter II of the PCT) must be established according to Rule 69.2 is: 14.03.2006</p>		

Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bertrand, F Telephone No. +49 89 2399-8606
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**WRITTEN OPINION OF THE INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY**

International application No.
PCT/GB2004/004637

IAP20 Receipt PCT/PTO 12 MAY 2006

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion is based on the international application in the language in which it was filed, unless otherwise indicated under this item.
 - This opinion is based on translations from the original language into the following language, which is the language of a translation furnished for the purposes of:
 - international search (under Rules 12.3 and 23.1(b))
 - publication of the international application (under Rule 12.4)
 - international preliminary examination (under Rules 55.2 and/or 55.3)
2. With regard to the **elements** of the international application, this opinion is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed"*):

Description, Pages

1-23	as originally filed
24	received on 12.09.2005 with letter of 09.09.2005

Claims, Numbers

1-31	as originally filed
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- a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.
- 3. The amendments have resulted in the cancellation of:
 - the description, pages
 - the claims, Nos.
 - the drawings, sheets/figs
 - the sequence listing (*specify*):
 - any table(s) related to sequence listing (*specify*):
- 4. This opinion has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
 - the description, pages
 - the claims, Nos.
 - the drawings, sheets/figs
 - the sequence listing (*specify*):
 - any table(s) related to sequence listing (*specify*):

**WRITTEN OPINION OF THE INTERNATIONAL
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International application No.
PCT/GB2004/004637

**Box No. V Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or
industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	1-31
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-31
Industrial applicability (IA)	Yes:	Claims	1-31
	No:	Claims	

2. Citations and explanations:

see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY
(SEPARATE SHEET)

PCT/GB2004/004637

JAP20 Rec'd PCT/PTO 12 MAY 2006

Re Item I

Basis of the report

The documents mentioned herein are numbered in accordance with the order they appear in the International Search Report.

The amendments filed by the Applicant on the 09.09.2005 comply with Article 34(2)(b) PCT insofar as they do not introduce any subject-matter which extends beyond the application as originally filed. They are thus admissible. The amendments consist in making clear which data of Table 5 (page 24) are obtained from the indicated information source.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The present invention relates to combustible material containing bifenthrin as active ingredients for killing mosquitoes.

It is assumed that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the document D1 (see Item VI below) could become relevant in the national/regional phase to assess whether claims 1-31 satisfy the criteria set forth in Article 33 PCT.

None of the documents D2-D4 disclose combustible material for dispensing bifenthrin. Novelty is thus acknowledged (Art.33(2)PCT).

The subject-matter of the present claims has 2 relevant aspects to be evaluated for assessing inventive step (Art.33(3)PCT): the fact that bifenthrin is used in fumigators instead of the classical volatile pyrethroids and the formulation parameters.

Having regard to the use of bifenthrin, the applicant submitted that the use of a non volatile pyrethroid in general was not disclosed at the relevant date and that bifenthrin would not have been considered for combustible materials since it decomposes at 170°C.

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D2 mentions some pyrethroids for use in smoke fumigants, e.g. cypermethrin, permethrin and deltamethrin, which the applicant acknowledged as non volatile. D3 discloses the possible simultaneous use of such non volatile pyrethroids and even of bifenthrin. D4 describes the use of bifenthrin for fumigation. The fact that D4 uses higher concentrations of bifenthrin is not relevant for the question of thermal stability. D4, Table 1 discloses clearly that 90% of the bifenthrin used in the combustible product is recovered from smoke. One skilled in the art would understand from D4 that, in spite of a known decomposition at 170°C, bifenthrin can be used with minimal loss of activity in combustible insecticides. Therefore, the alternative is obvious.

The applicant made clear with amendments of the header of Table 5 that the toxicity data were provided by himself rather than from the prior art. However, D5 (Comparative toxicity of permethrin and bifenthrin treated cloth fabric for *Anopheles farauti* and *Aedes aegypti*, S.P.Frances *et al.*, J. Am. Mosq. Control Assoc., 19(3):275-279, 2003) clearly discloses that a higher level of control of *A. Aegypti* is achieved with a lower amount of bifenthrin compared to permethrin. Therefore even the problem of providing an improved coil is solved in an obvious manner since the skilled artisan would have selected bifenthrin as a known more efficient insecticide.

Having regard to the formulation parameters, i.e. the presence of an accelerator, of a regulator/retardant, etc. the prior art provides sufficient information for their use. It is pointed out that each component is known for a specific role, that this role is the one for which it has been selected in the present invention and that this constitutes a juxtaposition of means which do not cooperate with/influence each other. As to the relative amounts of the components present, although the exact values can't be found as such in the cited prior art, it is within the artisan's skills to optimize these values. So far, no unexpected effect due to specific formulation parameters have been substantiated.

The present application does thus not fulfill the criteria of Article 33(3) PCT, because the claimed subject-matter does not involve any inventive step (Rule 65(1) and (2) PCT).

Re Item VI

Certain documents cited

**WRITTEN OPINION OF THE INTERNATIONAL
PRELIMINARY EXAMINING AUTHORITY
(SEPARATE SHEET)**

International application No.
PCT/GB2004/004637

Certain published documents

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (<i>valid claim</i>) (day/month/year)
WO 2004/031104	15.04.2004	02.10.2003	02.10.2002

SCIENTIFIC NOTE

COMPARATIVE TOXICITY OF PERMETHRIN- AND BIFENTHRIN-TREATED CLOTH FABRIC FOR *ANOPHELES FARAUTI* AND *AEDES AEGYPTI*¹

S. P. FRANCES,² K. WATSON^{3,4} AND B. G. CONSTABLE⁵

ABSTRACT. In this laboratory study, we applied 3 formulations of permethrin (Peregin® 500 EC, Dragnet® 500 EC, and Dragnet® 100 ME) and 2 of bifenthrin (Biflex® 10 ME and Talstar® 80 SC) to swatches of Australian military shirt fabric. The knockdown and mortality of *Anopheles farauti* and *Aedes aegypti* after exposure to treated fabrics were compared. The mortality of *An. farauti* exposed to permethrin-treated swatches for 3 min in World Health Organization test kits was 94.2–100% after initial treatment and fell to <28% after 2 cold water washes, and knockdown was <20% after 3 washes. The mortality of *An. farauti* exposed to bifenthrin-treated swatches was initially 100% and remained >55% after 3 washes, whereas knockdown was <25% after 2 washes. Mortality of *Ae. aegypti* exposed by tarsal contact to permethrin- and bifenthrin-treated fabrics was 84.8–100% prior to washing and fell to <21% and <40%, respectively, after 1 cold water wash. The ability of *Ae. aegypti* to obtain a blood meal through treated fabrics was variable, and a small percentage (0–6.1%) of mosquitoes obtained a blood meal through fabrics after initial treatment. The effect of cold water washing on the persistence of both chemicals in fabric by chemical assays showed that between 58% and 66% of both chemicals was lost from the test fabric after a single wash.

KEY WORDS *Anopheles farauti*, *Aedes aegypti*, bifenthrin, permethrin

The wearing of clothing treated with permethrin to protect people against mosquito vectors of disease has been advocated in recent years. Military forces deployed to areas where malaria, dengue, and arboviruses are endemic have used this method (Horosko and Robert 1996, Young and Evans 1998).

Earlier studies showed that a combination of the wearing of permethrin-impregnated military uniforms and application of repellents containing *N,N*-diethyl-3-methylbenzamide (deet) on the exposed skin provided the best protection against mosquito (Gupta et al. 1987, Harbach et al. 1990). In some instances, the wearing of permethrin-treated uniforms alone provided better protection against mosquitoes than did untreated uniforms, but this protection was less than that provided by the combination of permethrin-treated uniforms and topically applied deet (Schreck et al. 1984). This finding prompted 2 groups to evaluate the effectiveness

of permethrin-treated uniforms in protecting soldiers against malaria. In a trial in northeastern Thailand, the use of permethrin-treated uniforms did not reduce the incidence of malaria in Thai soldiers over a 6-month period (Eamsila et al. 1994). However, in a later study in Colombia, permethrin-treated uniforms provided better protection against malaria than untreated uniforms over a 4-week period (Sota et al. 1995).

Bifenthrin is a non-alpha cyano pyrethroid used against a range of agricultural pests and, recently, as an insecticide treatment for mosquito bednets (Hougaard et al. 2002). The chemical has a relatively low irritant and knockdown effect compared with permethrin and deltamethrin. Bifenthrin caused a higher mortality by allowing mosquitoes to rest on treated surfaces for longer periods (WHO 2001).

The aim of this laboratory study was to compare the knockdown and mortality of mosquitoes exposed to shirt fabric impregnated with 3 formulations of permethrin and 2 formulations of bifenthrin. Because pyrethroids are lost from clothing primarily as a result of laundering (Schreck et al. 1982), the persistence of the formulations in treated fabric after washing in cold water was also compared.

The following insecticide formulations were evaluated during this study: 1) Dragnet® 100 ME, containing 100 g/liter permethrin (25:75 cis:trans) as a micro emulsion, produced by FMC, Australia; 2) Dragnet® 500 EC, containing 500 g/liter permethrin (25:75 cis:trans) as an emulsifiable concentrate, produced by FMC, Australia; 3) Peregin® 500 EC, containing 500 g/liter permethrin (25:75

¹ This paper is published with the approval of the Director General Defence Health Service (Australia). The views of the authors do not purport to reflect the position of the Australian Defence Force or Department of Defence (Australia). Mention of a commercial product does not constitute an endorsement of the product by the Department of Defence (Australia).

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Table 1. Knockdown (\pm SE) after 60 min and mortality (\pm SE) after 24 h of *Anopheles farauti* after a 3-min exposure to cloth treated with insecticides.¹

Treatment	Number of cold water washes					
	0	1	2	3	2	3
	KD (%)	Mort (%)	KD (%)	Mort (%)	KD (%)	Mort (%)
No treatment	0	2.0 ± 2.0	0	2.0 ± 2.0	0	4.0 ± 2.5
Dragnet 100 ME	100	100	81.2 ± 7.8	46.6 ± 14.6	8.0 ± 2.0	4.4 ± 2.7
Dragnet 500 EC	100	100	88.4 ± 5.0	70.4 ± 4.9	28.0 ± 3.2	18.0 ± 3.8
Peregin 500 EC	100	94.2 ± 2.5	91.8 ± 3.8	63.8 ± 6.4	31.0 ± 5.1	20.2 ± 7.0
Talstar 80 SC	100	100	44.0 ± 8.1	97.8 ± 2.2	5.8 ± 2.4	27.2 ± 5.5
Biflex 10 ME	100	39.6 ± 4.3	95.8 ± 2.6	0	88.6 ± 5.4	18.8 ± 6.1
					55.8 ± 9.9	1.8 ± 1.8
					18.2 ± 3.2	70.6 ± 6.1
						59.8 ± 7.0

¹KD, knockdown; Mort, mortality.

cis : trans) as an emulsifiable concentrate, produced by Aventis, Australia; 4) Talstar[®] 80 SC, containing 80 g/liter bifenthrin as a suspension concentrate, produced by FMC, Australia; and 5) Biflex[®] 10 ME, containing 10 g/liter bifenthrin as a micro emulsion, produced by FMC, Australia.

Australian Defence Force Disruptive Pattern Camouflage Uniform shirt fabric, made of 50% cotton/50% polyester, was used. A total of 60 swatches from this material measuring 15 cm × 12 cm were prepared. The test insecticides were mixed with water at the following rates: 60 ml formulation/liter water for Dragnet 100 ME, 12 ml/liter for Dragnet 500 EC and Peregin, 2.5 ml/liter for Biflex 10 ME, and 6.25 ml/liter for Talstar 80 SC. The emulsion was mixed in a small hand-held sprayer and applied at a rate of 100 ml/m². The swatches were weighed before and after treatment to estimate the application rate of insecticide. Swatches were allowed to air dry in the laboratory before being wrapped in aluminum foil. The 5 test chemicals were applied to 10 swatches each, with an additional 10 swatches left untreated. Five swatches of each treatment and controls were used for exposure to mosquitoes, and five of each treatment were used for chemical analysis.

Mosquitoes used in the study were from colonies maintained in our laboratory in Brisbane. They were *Anopheles farauti* Laveran, 6–7-day-old nulliparous females from a colony established from Rabaul, Papua New Guinea, in 1972, and 2–6-day-old nulliparous female *Aedes aegypti* (L.) from a colony originally established from the Queensland Institute of Medical Research, in 1981.

A contact bioassay and a biting bioassay were conducted on the test fabrics. The contact bioassay used World Health Organization (WHO) susceptibility test kits, which allowed mosquitoes to be exposed to fabrics for short times. Five replicate groups of *An. farauti* females, each of 10 mosquitoes, were exposed for 3 min after initial treatment and then after each cold water wash. Test swatches were rolled and fixed into the test kit treatment cylinder with 2 metal clips. After exposure to the test fabrics, the mosquitoes were transferred to the holding cylinder of the WHO kit. The cylinders were placed into a polystyrene container and covered with moist cotton wool. The knockdown of mosquitoes was scored 60 min after exposure, and mortality was scored at 24 h. A mosquito was scored as knocked down if it was lying on its back or side and was unable to maintain flight after a gentle tap on the cylinder.

The biting bioassay involved placing 7–10 *Ae. aegypti* adults between the lid of a petri dish (8.5 cm diameter, 1.3 cm high) and a card. Three replicate groups of mosquitoes were tested for each treatment after initial treatment and after each cold water wash. For each biting test, the test fabric was held tightly over the stomach of a human volunteer, and then the card was removed to expose the mos-

Table 2. Biting and mortality (\pm SE) of *Aedes aegypti* after exposure to cloth treated with insecticides for 3 min.¹

Treatment	Number of cold water washes					
	0	1	2	3	Fed (%)	Mort (%)
No treatment	32.1 ± 6.6	6.7 ± 6.7	14.6 ± 5.5	5.0 ± 5.0	20.8 ± 15.0	3.3 ± 3.3
Dragonet 100 ME	6.1 ± 6.1	84.8 ± 8.0	6.4 ± 3.2	9.7 ± 0.3	3.7 ± 3.7	15.3 ± 9.7
Dragonet 500 EC	3.3 ± 3.3	97.0 ± 3.0	0	18.5 ± 5.0	0	5.6 ± 5.6
Peregrin 500 EC	0	93.0 ± 3.5	0	20.9 ± 1.3	0	3.3 ± 3.3
Talstar 80 SC	3.7 ± 3.7	100	9.1 ± 9.1	39.1 ± 6.6	4.2 ± 4.2	7.4 ± 3.7
Biflex 10 ME	3.3 ± 3.3	96.7 ± 3.3	7.5 ± 3.9	32.7 ± 24.2	7.4 ± 3.7	20.4 ± 8.6
Bare skin	—	—	47.0 ± 19.7	9.1 ± 9.1	23.3 ± 14.5	3.7 ± 3.7

¹ Mort, mortality.

quitoes to the fabric for 3 min. The card was then returned and the mosquitoes were liberated into a 250-ml cup. Sixty minutes later, the number of bloodfed mosquitoes and knockdown were recorded, and mortality was recorded at 24 h. The biting response of a sample of test mosquitoes was also assessed by exposing them to the bare stomach of the volunteer.

At the conclusion of each round of mosquito bioassays, all swatches were washed by hand in cold water containing 4 g/liter of a commercial washing powder (Septone, Blue Lustre). Each swatch was washed gently by kneading in detergent water for a total of 4 min, then rinsed twice with fresh water for 1 min per rinse.

A subsample of 3 replicate swatches (5 × 6 cm) of each treatment was obtained after initial treatment and after each cold water wash for chemical analysis of permethrin and bifenthrin. Because only 4 rounds of chemical tests were completed, some swatch material remained. The active ingredient was extracted from the swatches with 100% acetone in an ultrasonic bath for 10 min. The analysis was conducted by high-performance liquid chromatography fitted with a photodiode array detector with 85% acetonitrile as the mobile phase.

The knockdown and mortality of *An. farauti* after 3 min of exposure to cloth treated with 5 insecticides are shown in Table 1. Because of the variability, valid statistical analysis was not possible. The mortality of mosquitoes exposed to permethrin-treated swatches was 94.2–100% after initial treatment, and this fell to <28% after 2 cold water washes. Knockdown was initially 100% and fell to <25% after 3 washes. By contrast, the mortality of mosquitoes exposed to bifenthrin was >55% even after 3 cold water washes.

The biting of *Ae. aegypti* through cloth fabric is shown in Table 2. The number of mosquitoes feeding through untreated and treated fabric varied, but a small percentage of mosquitoes fed through treated fabrics after initial treatment.

The persistence of active ingredient in treated fabrics is shown in Table 3. In the current experiments, between 62% and 67% of permethrin was lost from swatches after a single cold water wash, and 79–82% was lost after 3 washes (Table 3). The effect of washing on bifenthrin-treated cloth was similar to permethrin and is also shown in Table 3. With Talstar 80 SC-treated cloth, 63.2% of the active ingredient was lost after the first wash and 85% after 3 washes. For Biflex 10 ME, 58.4% of active ingredient was lost after 1 wash and 82% after 3 washes. Despite the relatively low amount of active ingredient remaining in bifenthrin-treated swatches, there was relatively high mortality in *An. farauti* after exposure to the cloth for 3 min (Table 1).

This study has shown that fabric impregnated with both permethrin and bifenthrin was toxic to *An. farauti* and *Ae. aegypti* after initial treatment. However, 61–66% of permethrin and 58–63% of

Table 3. Mean concentration (\pm SE) of permethrin and bifenthrin in shirt fabric after initial treatment and after cold water washing.

Number of washes	Permethrin content (mg/m^2)			Bifenthrin content (mg/m^2)	
	Dragnet 100 ME	Dragnet 500 EC	Peregin 500 EC	Talstar 80 SC	Biflex 10 ME
0	633.0 \pm 32.6	617.0 \pm 22.0	644.0 \pm 10.1	38.1 \pm 0.9	58.2 \pm 1.2
1	214.3 \pm 3.2 (66.1) ^a	236.3 \pm 4.3 (61.7)	228.0 \pm 4.0 (64.6)	14.0 \pm 0.2 (63.2)	24.2 \pm 1.5 (58.4)
2	198.7 \pm 11.1 (68.6)	221.7 \pm 13.2 (64.1)	192.3 \pm 1.7 (70.1)	6.8 \pm 0.5 (82.2)	17.4 \pm 0.5 (70.1)
3	117.0 \pm 11.0 (81.5)	132.7 \pm 9.1 (78.5)	124.0 \pm 2.1 (80.7)	5.7 \pm 0.4 (85.0)	10.5 \pm 1.9 (82.0)

^a Numbers in parentheses show the percentage of active ingredient lost compared with the initial treatment.

bifenthrin were lost after the first cold water wash. After 3 washes, knockdown and mortality of *An. farauti* were low (<38%). Despite the loss of 82–95% of the active ingredient from fabric, the overall mortality of *An. farauti* exposed to bifenthrin-treated fabric was greater than 55%, even after 3 cold water washes. The biting of *Ae. aegypti* through treated fabric varied, but 0–6.1% of mosquitoes obtained blood through fabrics after initial treatment.

We thank K. L. Rowcliffe for laboratory assistance and R. D. Cooper for comments on the manuscript.

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Table 5 - Comparison of physical properties of bifenthrin with other pyrethroids

(Source of date relating to vapour pressure, melting point, boiling point and molecular weight: The Pesticide Manual, A World Compendium. 12th Ed. Editor C.D.S. Tomlin. British Crop Protection Council. The data relating to mortality is experimental data prepared by the inventors.)

	Vapour Pressure (mPa)	Melting Point (°C)	Boiling Point (°C)	Molecular Weight	% mortality of Ae. aegypti (15 mins exposure)
0.25% d-Allethrin	0.16 (21°C)	-	281.5	302.4	7
0.05% Bifenthrin	0.024 (25°C)	68-70.6	Decomp>170	422.9	94
0.228% d-Phenothrin	0.019 (21.4°C)	-	>290	350.5	24
0.035% Imiprothrin	0.0018 (25°C)	-	-	318.4	7
0.705% Permethrin	0.0025 (20°C)	34-35	200	391.3	55
0.3% Cypermethrin	0.0002 (20°C)	61-83	-	416.3	56
0.056% Bioresmethrin	18.6 (25°C)	32	Decomp>180	338.4	23
0.07% Deltamethrin	0.0000124 (25°C)	100-102	-	505.2	12
Blank coil					6
Untreated control					10

5

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as 10 illustrative and not restrictive.